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Neuronal Calcium Channel Inhibitors

SYNTHESIS OF ω-CONOTOXIN GVIA AND EFFECTS ON ^{45}Ca UPTAKE BY SYNAPTOSOMES*

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Jean Rivier‡, Robert Galyean‡, William R. Gray§, Aryan Azimi-Zonooz§, J. Michael McIntosh§, Lourdes J. Cruz§¶, and Baldomero M. Olivera§‖

From the ‡Clayton Foundation Laboratories for Peptide Biology, Salk Institute, San Diego, California 92138, the §Department of Biology, University of Utah, Salt Lake City, Utah 84112, and the ¶College of Medicine and Marine Sciences Institute, University of the Philippines, Manila, Philippines

We previously described a 27-amino acid peptide neurotoxin from the venom of *Conus geographus*, ω-conotoxin GVIA, which inhibits neuronal voltage-activated calcium channels. In this paper we describe the total synthesis of ω-conotoxin GVIA and demonstrate that it efficiently blocks voltage-activated uptake of ^{45}Ca by standard synaptosomal preparations from chick brain. Dihydropyridines do not block ^{45}Ca uptake under these conditions. Thus, the ω-conotoxin-sensitive, but dihydropyridine-insensitive uptake of $^{45}Ca^{2+}$ by chick brain synaptosomes serves as a functional assay for a Ca channel target of ω-conotoxin.

The use of synthetic GVIA should rapidly accelerate our understanding of the molecular biology of Ca^{2+} channels and their role in neuronal function.

Voltage-sensitive calcium channels are critical components of neurons. Their role in neurotransmitter release is well established (1, 2). In addition electrophysiological work has indicated that calcium channels are essential for the generation of the complex firing patterns that are found in certain neurons (see for example Ref. 3). For this reason a biochemical characterization of calcium channels in neuronal tissue should be an important step toward understanding neuronal function at the molecular level.

To this point the major probes used for studying neuronal calcium channels have been the dihydropyridines (4, 5), and a large number of studies have been carried out using these agents. However, although the dihydropyridines have well-defined effects on voltage-sensitive Ca^{2+} channels in muscle, their effects on neuronal tissue have been complex, and the interpretation of the observed effects has been controversial.

Recently, we described a new neurotoxin probe for neuronal calcium channels, ω-conotoxin GVIA from the venom of the fish-hunting cone snail, *Conus geographus* (6, 7). This 27-amino acid peptide (Fig. 1) has been shown to block neuronal calcium channels in a variety of preparations (8).[1,2] However, the use of this promising agent for biochemical studies has been hampered by two factors. First, the availability of the toxin has been very limited because it has to be purified from the venom of rather small snails. Second, although we have developed a binding assay for ω-conotoxin GVIA (11), functional assays for its effects on voltage-activated calcium currents in neuronal tissue have been confined to electrophysiological methods which measure the response of only one cell or a small group of cells. There has been no convenient way to assess ω-conotoxin effects on Ca^{2+} channel activity in the same preparations in which ω-conotoxin binding is assayed.

The results reported in this communication should alleviate these problems. First, the chemical synthesis of ω-conotoxin GVIA is described, creating much more substantial amounts of toxin for biochemical studies. Second, we demonstrate that ω-conotoxin GVIA inhibits voltage-dependent uptake of ^{45}Ca by chick brain synaptosomes prepared by standard methods. Under the same conditions, the dihydropyridines do not inhibit.

EXPERIMENTAL PROCEDURES

Synthesis of ω-Conotoxin GVIA—All *t*-butyloxycarbonyl amino acids were of the L-configuration except for *t*-butyloxycarbonyl-Gly and were purchased from BACHEM, Torrance, CA. *t*-Butyloxycarbonyl amino acids included: (*S*-paramethoxybenzyl), Lys (2-chlorobenzyloxycarbonyl), Ser (*O*-benzyl), Hyp (*O*-benzyl), Thr (*O*-benzyl), Tyr (2-bromobenzyloxycarbonyl) and Arg (Tosyl). Asparagine was incorporated into the peptide with an unprotected side chain. Stepwise buildup of the peptide on paramethylbenzhydrylamine resin (12) (4 g:0.43 meq/g) was done automatically on a Beckman 990B synthesizer using previously reported protocols (13) and 1.25 eq of protected amino acid/g of resin. Trifluoroacetic acid (60% in CH_2Cl_2 + 2% ethanedithiol) was utilized for deblocking; triethylamine (10% in CH_2Cl_2) was used for neutralization; resin washing was accomplished by application of isopropyl alcohol (1% ethanedithiol) after trifluoroacetic acid treatment, and by methanol or dichloromethane at the other steps. Couplings (100–140 min) were mediated by diisopropylcarbodiimide in either dichloromethane, dimethylformamide, or mixtures thereof depending upon the solubility of the respective amino acid derivatives. *t*-Butyloxycarbonyl-Asparagine, as previously noted, was coupled unprotected in dimethylformamide in the presence of 2 eq of 1-hydroxybenzotriazole. Recouplings were automatically performed at $Arg^{25,17}$, Lys^{24}, Hyp^{21}, $Asn^{20,14}$, Cys^{16}, and $Ser^{9,3}$. A final peptide resin (11.2 g) was obtained. The peptide was released from the peptide resin (4.0 g) as the COOH-terminal amide by treatment with distilled anhydrous HF (75 ml) in the presence of *p*-cresol (10 g) at 0 °C for 30 min. After removal of the HF under reduced pressure, the resin was washed portionwise with 300 ml of diethyl ether. The peptide was quickly extracted from the resin with distilled water and diluted to 3.5 liters with chilled distilled water. The solution was adjusted to pH 7 with NH_4OH, and allowed to slowly stir and air oxidize at 4 °C for 2 days. The peptide solution, which initially gave a positive Ellman test with 5,5′-dithiobis-(2-nitrobenzoic acid), at this point showed no evidence of free sulfhydryl groups. After acidification of the solution to pH 5 with acetic acid, the peptide was concentrated by passage through a column containing approximately 75 ml of Bio-Rad Bio-Rex-70 cation exchange resin (H^+ form). After extensive washing with distilled H_2O (300 ml) and 5% aqueous acetic

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‖ To whom correspondence should be addressed.

[1] E. W. McClesky, A. P. Fox, D. Feldman, L. J. Cruz, B. M. Olivera, R., W. Tsien, and D. Yoshikami, unpublished data.

[2] D. H. Feldman, B. M. Olivera, and D. Yoshikami, unpublished data.

TABLE I
Characterization of conotoxin GVIA

[α]D[a]	HPLC analysis[b]					
	Solvent system		Flow rate	Gradient	Retention volume	% purity
	A	B				
			ml/min		*ml*	
−18.4 °C	TEAP 2.25	30% CH_3CN; 70% A	2	20% B (20 min) − 40% B	27.0	>98
	0.1% Trifluoroacetic acid	30% CH_3CN in 0.1% TFA	2	36% B (20 min) − 56% B	32.2	>98

[a] c = 0.5; 1% HOAc; 20 °C.
[b] UV monitoring was done at 210 nm. 0.2 absorbance units at full scale. Column was Vydac (0.46 × 25 cm) packed with C_{18}, 5-μm particles, 300 Å pore size.

acid (300 ml), the peptide was removed from the resin by the addition of 50% aqueous acetic acid (300 ml). The peptide solution was concentrated to a pale oil on a rotary evaporator and immediately taken up in distilled H_2O, shell frozen, and lyophilized to a white powder (yield, 930 mg). The crude peptide was purified using preparative HPLC[3] techniques as previously described (14); the gradient of acetonitrile applied to the preparative cartridge in TEAP-2.25 was 0–15% in 1 h, with a flow rate of 100 ml/min. Analysis of the generated fractions was achieved using isocratic conditions (15% acetonitrile in 0.1% trifluoroacetic acid) on a 5-μm Vydac C_{18} column. Desalting was carried out using an acetonitrile gradient from 0 to 25% in 0.1% trifluoroacetic in 45 min. Highly purified fractions were pooled and lyophilized yielding conotoxin G-VIA as the trifluoroacetate (25.6 mg). Results of the HPLC analysis of this fraction are given in Table I along with the optical rotation in 1% AcOH. Amino acid analysis gave the following ratios with expected values in parentheses: Asp (2), 2.61 including presence of Hyp (3); Thr (2), 1.91; Ser (6), 5.62; Gly (1), 1.00; Cys (6), 5.56; Tyr (3), 3.04; Lys (2), 2.02; Arg (2), 2.20.

FAB Mass Spectrometry—Positive ion FAB mass spectra were taken with a Jeol HX-100 HF double-focusing mass spectrometer operating at 5 kV accelerating potential and using a 3 kV xenon atom beam (cam). 1–10 nmol of peptide were dissolved in ~1 μl of 5% aqueous HOAc and 1 μl of glycerol on a 1.5 × 6-mm stainless steel sample stage. Spectral data were collected and mass assigned using a Jeol DA5000 data system. Mass assignments are accurate to ±0.2 atomic mass unit. The spectrum showed a protonated molecular ion (MH^+) at $m/z = 3036.0$, corresponding to the calculated monoisotopic fully oxidized peptide amide of 3036.2.

Sequence Verification—A sample of peptide was reduced and carboxymethylated, and analyzed for 27 cycles of degradation in a Beckman 890D sequencer, as previously described (6).

A second synthetic strategy using the acetamidomethyl protecting group for cysteine was also successful. Synthesis proceeded essentially as described above. The linear acetamidomethyl-Cys protected peptide was obtained after HF cleavage and was purified using preparative HPLC. Amino acid analysis of this material gave the expected ratios. Removal of the acetamidomethyl protecting group was achieved using $Hg(OAc)_2$ and previously reported protocols (15). Cyclization using air oxidation was carried out as described above. Purification by semipreparative HPLC yielded the desired product. Because yields of purified material were not significantly different from that obtained in our first protocol using *S*-paramethoxybenzyl Cys, which is considerably simpler, we favor the former synthetic scheme for the synthesis of GVIA.

Biological Assay—Intracerebral injection of 11–14 g mice with ω-conotoxin resulted in a persistent tremor as described previously (6). At low doses (1–2 nmol/kg), mild tremors could be induced by holding mice by their tails and rotating slowly ("tail test").

Preparation of Synaptosomes—The method used was based on that described by Krueger *et al.* (16). Unless otherwise specified, all centrifugation was for 1 min using an Eppendorf 5412 centrifuge at 15,000 rpm (15,600 × *g*). In a typical preparation of synaptosomes to be used in calcium uptake experiments, 18 brains were removed from 2-day-old chicks and homogenized in 80 ml of ice-cold 0.32 M sucrose, 0.5 mM HEPES, pH 7.4 using a glass-Teflon homogenizer (clearance 0.175 mm) at 850 rpm (12 strokes). The homogenate was centrifuged at 3600 rpm for 10 min in a Sorvall SS-34 rotor. The supernatant was retained, and the pellet was resuspended in 40 ml of buffered 0.32 M sucrose and homogenized with 4 strokes. The supernatants were combined and centrifuged at 3600 rpm for 10 min and the pellet was discarded. The supernatant was then centrifuged at 12,500 rpm for 20 min, retaining the pellet. A dense clump of mitochondria had sedimented to the bottom of the tube; the looser pellet above this layer was carefully scraped off, and was then suspended in 40 ml of buffered 0.32 M sucrose and centrifuged at 12,500 rpm for 20 min. The supernatant was discarded. The pellet was resuspended in 30 ml of buffered 0.32 M sucrose. Aliquots (10 ml) were layered onto 20 ml 0.8 M sucrose, 5 mM Na HEPES, pH 7.4. Additional buffered 0.32 M sucrose was layered to fill the tube. The tubes were centrifuged at 8,300 rpm in a SW 27 swinging bucket rotor for 30 min. The 0.32 M sucrose layer and the interface were discarded. The 0.8 M layer was saved and the pellet was discarded. To the 0.8 M layer, approximately 700 ml of synaptosomal saline (S-saline) (145 mM NaCl, 5 mM KCl, 1.4 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10 mM glucose, and 20 mM Tris-HEPES, pH 7.4) was added in 1–5-ml portions over a period of 20 min. The mixture was centrifuged at 8,000 rpm in a GSA rotor for 8 min. The pellets were combined and resuspended in 55 ml of S-saline. Aliquots (0.5 ml) of this preparation were centrifuged, and the pellets were used for individual reactions.

^{45}Ca Uptake—The method used was based on that of Blaustein (17). To the synaptosomal pellet above, 45 μl of either toxin, dihydropyridine, or control buffer (S-saline + 0.2 mg/ml lysozyme) was added; the mixture was then completely resuspended and kept on ice for 30 min. An S-saline solution containing 10 mM $CaCl_2$ (5 μl) was added, the mixture was vortexed and incubated for 15 min at 30 °C. At the end of this incubation, 0.1 ml of either low potassium ^{45}Ca (0.2 mCi/ml ^{45}Ca dissolved in S-saline + 1 mM $CaCl_2$) or high potassium ^{45}Ca (0.2 mCi/ml ^{45}Ca in S-saline + 1 mM $CaCl_2$, with 137 mM KCl replacing NaCl) solution was added. After 1 s of incubation, the reaction was terminated by adding 0.05 ml of an EGTA solution (120 mM NaCl, 5 mM KCl, 30 mM EGTA-Tris, pH 7.6) followed by 0.6 ml of S-saline. The reaction mixture was centrifuged, the supernatant aspirated, and 0.8 ml of sodium wash (S-saline + 1 mM $CaCl_2$ without glucose or NaH_2PO_4) added to the pellet (without mixing). The supernatant was aspirated, and the pellet was resuspended in 0.8 ml of sodium wash with vigorous mixing. The mixture was recentrifuged and the sodium wash was repeated. After one more centrifugation, the supernatant was aspirated, and the pellet was resuspended in 0.4 ml of 1 N sodium hydroxide and incubated for 8 min at 68 °C. After vigorous mixing, it was incubated for an additional 8 min, followed by centrifugation. In order to determine radioactivity in the solutions, 0.066 ml of the sodium hydroxide mixture was added to 0.1 ml of 0.67 N HCl containing 2 mM calcium chloride and 4 ml of aquasol scintillation fluid.

In some preliminary experiments with crude synaptosomal fractions, filtration was used with results qualitatively similar to the centrifugation assay. A number of uptake experiments were also performed in S-saline in which NaCl was replaced by choline chloride; however, in this medium, depolarization-induced ^{45}Ca uptake by the chick brain synaptosomes was lower (<50%) than under the standard conditions above.

RESULTS

Synthesis of ω-Conotoxin GVIA—The synthesis of ω-conotoxin GVIA was carried out as described under "Experimental

[3] The abbreviations used are: HPLC, high pressure liquid chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; S-saline; synaptosomal saline; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; TEAP, triethylammonium phosphate.

C K S Hy G S S C S Hy T S Y N C C R S C N Hy Y T K R C Y –NH_2

FIG. 1. **Sequence of ω-conotoxin GVIA (from Olivera *et al.* (6)).**

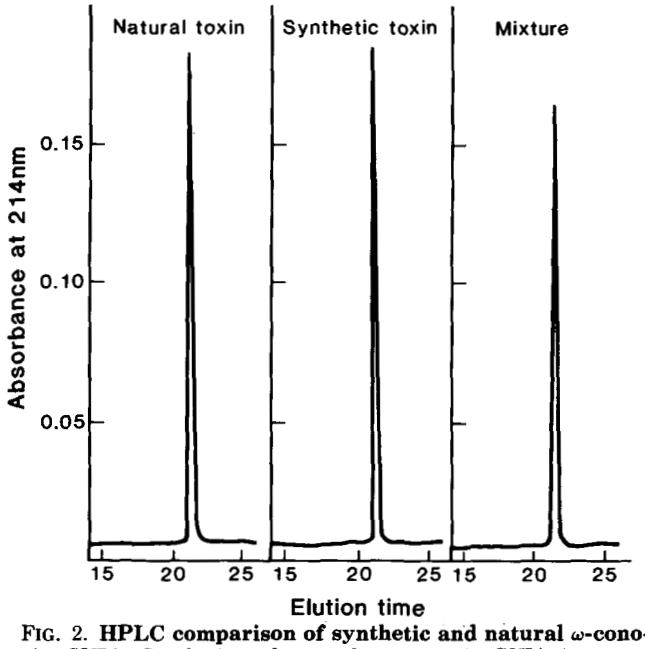

FIG. 2. **HPLC comparison of synthetic and natural ω-conotoxin GVIA.** Synthetic and natural ω-conotoxin GVIA (~1 nmol each), as well as an equimolar mixture (~0.45 nmol each) were chromatographed at 25 °C on a Vydac C_{18} analytical column. The peptides were eluted using 0.1% trifluoroacetic acid as solvent, applying a linear gradient from 9–28% (v/v) acetonitrile at a flow rate of 1 ml/min.

Procedures." The synthetic material was compared to natural ω-conotoxin GVIA. Protein sequencing of the synthetic material yielded the expected sequence, shown in Fig. 1. No significant "preview" of amino acids was observed, setting an upper limit of 1% on contamination by shortened peptides arising from incomplete reactions in synthesis. The synthetic toxin was analyzed by reverse phase HPLC using two different solvent systems. The results in the trifluoroacetic acid system are shown in Fig. 2. In all cases, the elution times of the synthetic material were identical to those of authentic natural toxin, and a single sharp peak was seen in both chromatographic systems upon mixing synthetic and natural toxins.

Biological activity of the synthetic material was compared to natural toxin as described under "Experimental Procedures." By these tests, synthetic and natural material were indistinguishable. Both elicited "shaker" symptoms in mice at higher doses (>2 nmol/kg), and at lower doses (~1 nmol/kg), tail test symptoms were elicited ("Experimental Procedures"). For both synthetic and natural toxins, a characteristic posture with back legs spread was assumed after the toxin was injected.

In addition, we have derivatized the synthetic toxin by iodination, and the iodinated derivatives of the synthetic toxin are identical in their elution times to iodinated derivatives of the natural toxin (results not shown). Thus, by all criteria applied, the synthetic material is identical to authentic natural toxin.

Effect of ω-Conotoxin GVIA on Chick Brain Synaptosomes—Chick brain synaptosomes were prepared by standard methods ("Experimental Procedures"). The purified synaptosomal preparation was then tested for voltage-sensitive ^{45}Ca uptake, and the effects of ω-conotoxin and nitrendipine (a dihydropyridine calcium channel antagonist) were evaluated. Results of three different experiments are shown in Table II. When ^{45}Ca was added with low K^+ (5 mM), a low constant level of ^{45}Ca was taken up by the synaptosomal vesicles. However, when ^{45}Ca was added in the presence of depolarizing concentrations of potassium (137 mM), ^{45}Ca uptake was significantly higher. This depolarization-induced uptake was greatly reduced (>90% inhibition) by predepolarization.

The depolarization-induced ^{45}Ca uptake was inhibited by ω-conotoxin (Table II). However, little or no inhibition was seen when nitrendipine, a dihydropyridine calcium channel inhibitor was added. The concentration dependence of ω-conotoxin effects is shown in Fig. 3. It is clear that ω-conotoxin is an effective inhibitor of $^{45}Ca^{2+}$ uptake by chick brain synaptosomes even at nanomolar concentrations. Under the present experimental conditions, 50% inhibition is achieved at 10^{-8} M ω-conotoxin, and almost total inhibition (>90%) is achieved at the highest concentration tested. In contrast, even at 10^{-4} M nitrendipine inhibition is marginal (see Table II, experiment 3).

The observed ^{45}Ca uptake could have occurred as a consequence of processes other than entry through voltage-sensitive Ca channels: Na^+-Ca^{2+} exchange is postulated to contribute significantly under certain conditions. We minimized nonchannel processes by monitoring short pulses (1 s) of ^{45}Ca. If a longer uptake period (*i.e.* 2 min) is allowed, ^{45}Ca uptake increases approximately 4-fold, but most uptake is not ω-conotoxin sensitive. Short pulses measure the "fast" component of Ca^{2+} uptake, generally attributed to voltage-sensitive Ca^{2+} channels; the "slow" component is primarily assigned to nonchannel processes (reviewed in Ref. 5). Another standard method used to minimize the latter is Na^+ replacement by choline; we found that ω-conotoxin inhibition of Ca uptake in choline medium still occurred (>60% inhibition of ^{45}Ca uptake).

The amount of voltage-dependent ^{45}Ca taken up by synaptosomes varies considerably from one synaptosomal preparation to the next (Table II). The functional voltage-sensitive calcium channels appear to have a half-life of only a few hours under the conditions being used (see Table III); in experiment 2, Table III, ~90% of voltage-dependent uptake was lost when the synaptosomal preparation was incubated in an ice bucket overnight. Variation in preparation times may partially ac-

TABLE II

^{45}Ca uptake measurements on chick brain synaptosomes

^{45}Ca uptake measurements were carried out on chick brain synaptosomes as described under "Experimental Procedures." The data points are average of three determinations with the standard deviation indicated. Each experiment was carried out on a different synaptosomal preparation.

^{45}Ca uptake	cpm
Experiment 1	
Low K^+, control	680 ± 120
High K^+, control	2340 ± 380
High K^+, + nitrendipine (10 μM)	2880 ± 380
High K^+, + ω-conotoxin (13.3 μM)	820 ± 200
Experiment 2	
Low K^+, control	270 ± 20
High K^+, control	1120 ± 150
High K, + nitrendipine (10 μM)	1170 ± 240
High K, + ω-conotoxin (13.3 μM)	470 ± 60
Experiment 3	
Low K^+, control	920 ± 50
High K^+, control	6250 ± 1300
High K^+, + nitrendipine (100 μM)	5910 ± 690
High K^+, + ω-conotoxin (13.3 μM)	2020 ± 270
Low K^+, + ω-conotoxin (13.3 μM)	930 ± 130

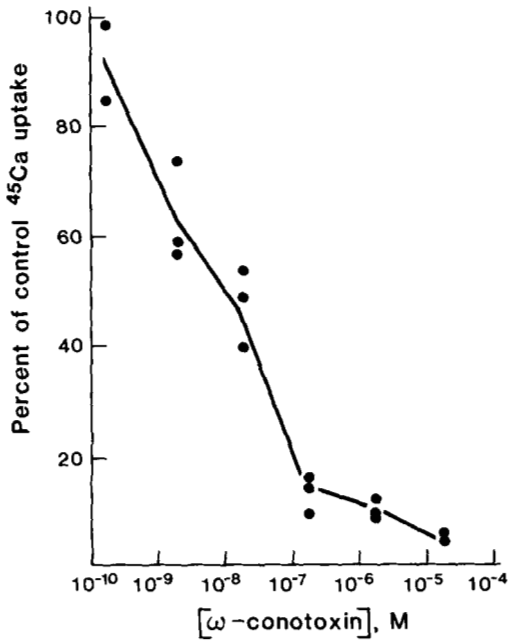

FIG. 3. **Concentration dependence of ω-conotoxin inhibition of ^{45}Ca uptake by chick brain synaptosomes.** ^{45}Ca uptake was measured as described under "Experimental Procedures." The synaptosomes were preincubated with ω-conotoxin at the concentrations indicated for 30 min before ^{45}Ca uptake was measured. In this experiment, the actual values for ^{45}Ca uptake were: high K control, 6000 cpm; low K control 1280 cpm. Thus, the difference between these two values, 4720 cpm was set as 100% of voltage activated ^{45}Ca uptake. Each experimental point for ^{45}Ca uptake in the presence of ω-conotoxin with the low K control (1280 cpm) subtracted out is shown by the *circles*; the *line* is drawn to the average of these points at each ω-conotoxin concentration.

TABLE III

Dependence of ^{45}Ca uptake on age of preparation

Experiments were carried out on two different synaptosomal preparations. Time is measured from the time the chicks were killed.

	H after dissection	^{45}Ca uptake			Net voltage-sensitive uptake	% ω-conotoxin inhibitable
		Low K	High K	High K + ω		
		cpm				
Experiment 1	11	1250	4930	1490	3680	93
	14	650	2120	670	1470	97
Experiment 2	16.7	790	5590	1960	4870	78
	28.5	490	1000	590	510	83

count for the inconstancy of ^{45}Ca uptake by different synaptosomal preparations, although other factors during synaptosome preparation may also influence $^{45}Ca^{2+}$ uptake by the final preparation. However, ω-conotoxin inhibition, and the lack of inhibition by dihydropyridines is seen reproducibly in all preparations.

It should be noted that although ω-conotoxin inhibitable ^{45}Ca uptake sites are labile (Table III), ω-conotoxin-binding sites are stable (results not shown). Thus, while voltage-activated calcium channels are rapidly inactivated in synaptosomal preparations, the results suggest that ω-conotoxin can still bind the inactivated calcium channels.

DISCUSSION

Calcium channels are a central, indispensable component of neuronal function. At the present time, voltage-activated Ca channels are the only system known to transduce electrical signals into biochemical events: for an electrical signal to result in an intracellular change a rise in cytoplasmic calcium levels must first take place. However, despite their key role in neuronal function, little progress has been made in the biochemical characterization of such voltage-sensitive calcium channels. The results described in this report establish that ω-conotoxins are a useful probe for these important neuronal molecules.

Calcium channels have been studied effectively in skeletal muscle and in cardiac tissue. The most commonly used agents for dissecting these channels in muscle are the dihydropyridine drugs such as nitrendipine. Purification of voltage-sensitive calcium channels from skeletal muscle T tubules was accomplished by using dihydropyridine binding as an assay (18, 19). However, the dihydropyridines have been relatively ineffective in studying calcium channels from neuronal tissue. In fact, there is increasing experimental evidence that calcium channels are probably different in neuronal and in muscle tissue, even though electrophysiologically similar calcium channel types may be found in both tissues (see for example Refs. 9 and 10).

Electrophysiological and pharmacological evidence strongly indicates that several different calcium channel types exist in neuronal tissue. The most direct evidence has been provided by Tsien and co-workers (10),[1] who have used single channel-recording techniques to characterize three different calcium channel types from embryonic chick dorsal root ganglion cells in culture. These three calcium channel types, which have been termed L, T, and N channels, differ in their electrophysiological characteristics, both with respect to their conductance once the channel is open, and to their activation and inactivation characteristics (10). In addition, the channels can be distinguished pharmacologically: L channels are affected by both the dihydropyridines and ω-conotoxin; N channels are effectively inhibited by ω-conotoxin but not by the dihydropyridines. The T channels are relatively resistant to inhibition by both the dihydropyridines and ω-conotoxin; with the latter, only a transient inhibition is observed.[1]

Although the level of analysis is far less sophisticated than the most advanced electrophysiological methods, the use of synaptosomal preparations allows bulk assays of ion channels. Most workers find that the majority of calcium currents in synaptosomes of neuronal origin appear to be refractory to dihydropyridine inhibition, a result we obtained in the chick brain preparation (for a review, see Ref. 5). Our results demonstrate that ω-conotoxin is effective in abolishing voltage-activated ^{45}Ca entry into chick brain synaptosomes. We conclude that in this synaptosome preparation, the voltage-activated entry of ^{45}Ca is mediated mainly by N-like channels, which are ω-sensitive but dihydropyridine-insensitive. Whether these will prove to be identical to the N channels that have been described from embryonic chick dorsal root ganglion cells in culture (10) remains to be established. Since synaptosomes come primarily from nerve endings, the results suggest that N-type channels may be the predominant channel in the presynaptic termini of nerves.

In addition to characterizing the nature of voltage-sensitive calcium channels in synaptosomes, the experiments described here provide a convenient assay both for ω-conotoxin and active target channels. Previously, we described the binding of ω-conotoxin to synaptosomal preparations. The present experiments provide a functional correlate to binding studies. The development of a relatively simple functional assay, and the availability of chemically synthesized ω-conotoxin GVIA should significantly increase the potential of this peptide neurotoxin for the biochemical characterization of neuronal calcium channels.

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